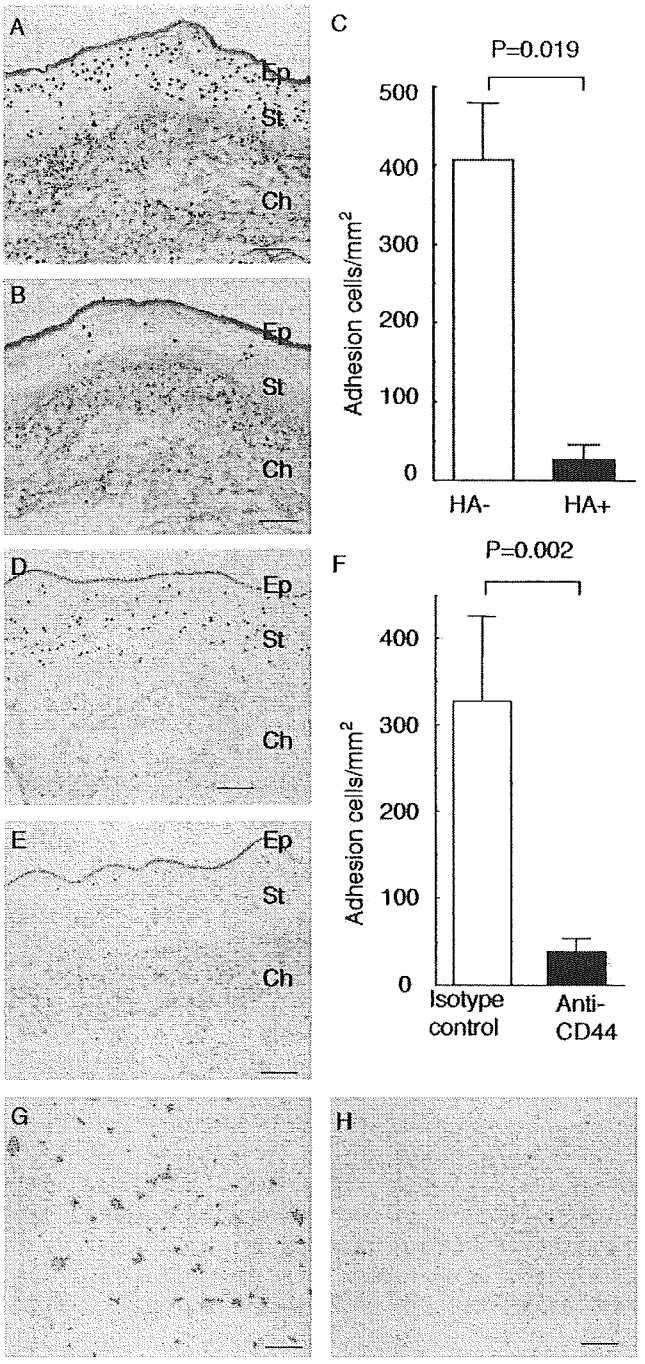

FIGURE 3. Molt 4 adhesion to AM sections was significantly inhibited by preincubation with soluble HA (1 mg/mL) (B) compared with control (A, C). (HA^-, n = 8; HA^+, n = 6). Adhesion of Molt 4 to AM was also inhibited by anti-CD44 mAb (E) but not by isotype control (D, F) (n = 10). Anti-CD44 mAb also inhibited Molt 4 adhesion to HA-immobilized glass slides (H) compared with control (G). Ep, amniotic epithelium; St, stroma of AM; Ch, chorion. The bar represents 100 μm.

Adhesion Assay of PBMC

Both LPS and TNF-α increased the adhesion of PBMC to the AM stroma but not to amniotic epithelium and chorion (Fig. 4D–F). PBMC binding assays were repeated using HA-coated glass slides. As with the AM adhesion assay, stimulation with LPS or TNF-α increased PBMC adhesion to immobilized HA (Fig. 4F–H). PBMC adhesion induced by LPS or TNF-α treatment was also blocked by anti-CD44 mAb to 11.5% and 7.7% of control, respectively (Fig. 5, $P = 0.003$, $P = 0.002$). To compare adhesion with other glycosaminoglycans, PBMC binding assays were repeated using chondroitin sulfate (CS)-coated glass slides. PBMC treated with LPS or TNF-α did not show an increase in adhesion to immobilized CS (Fig. 4I–K), suggesting that HA–CD44 interaction is the main mechanism involved in lymphocyte adhesion to AM.

DISCUSSION

High-molecular-weight HA (approximately 1.67×10^6) was detected in high levels within the stroma of AM but not in the amniotic epithelium. The pattern of HA distribution corresponded with the collagen-rich zones of AM[21] and consisted of typical fibrous connective tissue with a high concentration of type IV and V collagen.[1] Our results were consistent with a previous report measuring HA distribution in AM.[22] Various experimental studies have shown that the antiinflammatory effects of high-molecular-weight HA are associated with its scavenging of free radicals,[23] inhibition of cytokine production,[24] or suppression of elastase release from activated peritoneal leukocytes.[25] In a previous report, supplementation of dialysis fluid with high-molecular-weight HA reduced the intraperitoneal inflammatory reaction in rats maintained for 1 mouth on peritoneal dialysis.[26] The high-molecular-weight HA in AM may also exert such effects; however, physical sequestration alone, by trapping inflammatory cells, may also have antiinflammatory effects.

Pathology of clinical samples after AM patching to the ocular surface revealed the entrapment of inflammatory cells of monocyte/macrophage lineage and lympocytes throughout the thickness of the stroma.[12,22] An approximately equal ratio of $CD4^+$ and $CD8^+$ cells were found, indicating that both types of T lymphocytes were present. We found that CD44, expressed on Molt 4 and PBMC when stimulated with IL-2 and IFN-γ,[27] was required for adhesion to both HA-coated slides and fixed AM stroma sections. Although both CD44 and the β1 integrin heterodimers play a role in mediating the adhesion of ovarian carcinoma cells to mesothelial cells,[28] our previous study indicated that adhesion of Molt 4 was not inhibited by blocking the adhesion molecules β1 and β2 integrins.[12]

The abundance of the high-molecular-weight form of HA may be crucial to the physiological effects observed following AM patching of the inflamed ocular surface.

The entrapment of inflammatory cells may explain the antiinflammatory effects often observed following AM patches. As presented in our previous study, in a clinical situation, infiltrating cells are found throughout the AM stroma and not only at the junction of AM and cornea/sclera.[12] Most of the infiltrating lymphocytes and/or monocytes were TUNEL positive in the AM obtained from clinical samples.[12] Fetal membranes express FasL, by which the fetus is afforded protection against cytolytic actions of lymphocytes from the mother.[29–32] Whether the apoptosis process is an active effect exerted by AM remains to be clarified because there is the possibility that lymphocytes simply underwent a physiologic course of apoptosis while being trapped within the AM. Because none of the histologic samples showed infiltrating cells invading the basement membrane, most of the cells seem to have been sequestered from the ocular surface lying directly beneath the AM.[12] There is also the possibility that the AM also acts as a physical barrier, as with contact lenses, to protect the ocular surface from inflammatory cells in the tear film.

AM has the ability to suppress allo-reactive T cells in vitro,[33] an effect that may be mediated by secretory factors such as PGE_2,[34] HLA-G,[35] and FasL.[29–32] Because high-molecular-weight HA is also associated with inhibition of cytokine production,[24] HA in AM stroma may regulate both T_H1(IL-2 and IFN-γ) and T_H2 (IL-6 and IL-10) types of cytokine production.[33]

In summary, HA was present in high levels in the stroma of AM. Our data demonstrate that HA–CD44 interaction plays an important role in the adhesion of inflammatory cells, including lymphocytes, to AM stroma. Although further studies are required to elucidate the molecular events involved, entrapment of inflammatory cells may explain some of the clinical effects observed in the use of AM in ocular surface reconstruction.

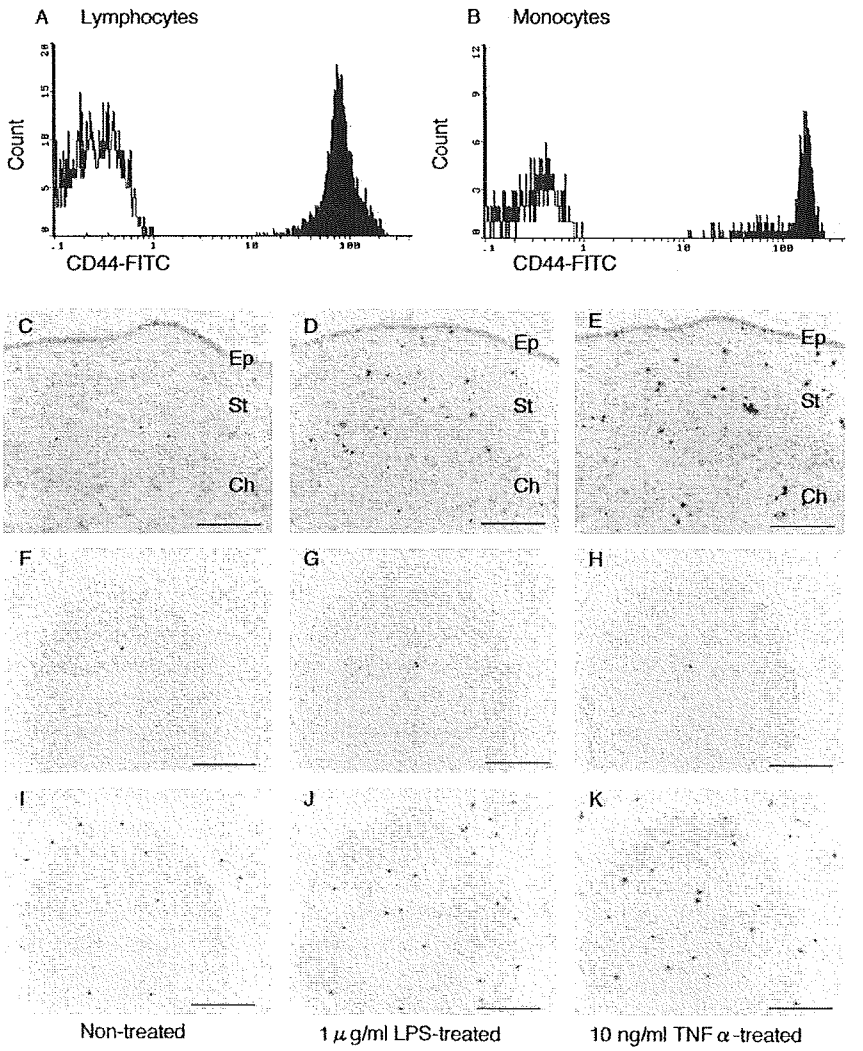

FIGURE 4. PBMC expressing CD44 (A, B) were used in an adhesion assay to AM (C, E), immobilized chondroitin sulfate (CS) (F, H), and immobilized HA (J, K). Activation of PBMC by preincubation with 1 μg/mL LPS or 10 ng/mL TNF-α for 72 hours caused increased adhesion in AM and HA-coated slides but not in CS-coated slides. Ep, amniotic epithelium; St, stroma of AM; Ch, chorion. The bar represents 100 μm.

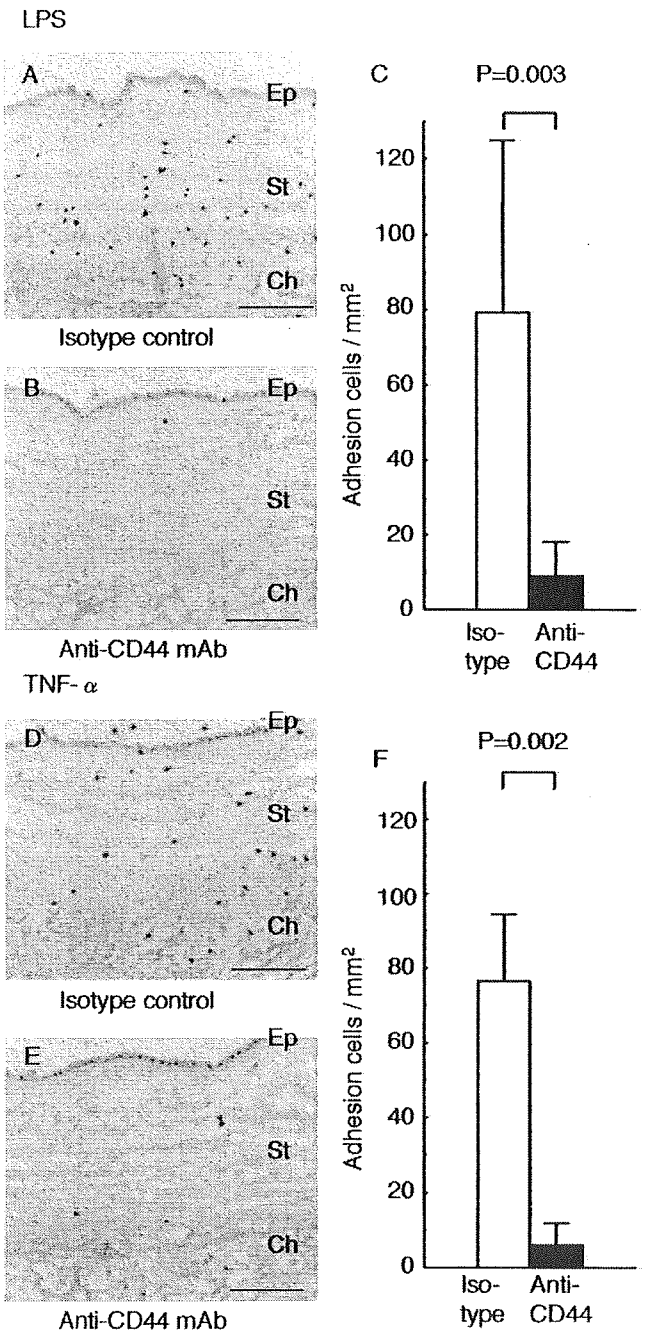

FIGURE 5. Adhesion of LPS-treated (A) and TNF-α–treated (D) PBMC to AM was inhibited by the anti-CD44 mAb (B, E). Anti-CD44 mAb significantly inhibited adhesion of PBMC activated by both LPS (C) and TNF-α (F) (n = 10 each). Ep, amniotic epithelium; St, stroma of AM; Ch, chorion. The bar represents 100 μm.

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The Application of a New Continuous Functional Visual Acuity Measurement System in Dry Eye Syndromes

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• PURPOSE: To evaluate the efficacy of a new continuous functional visual acuity measurement (FVAM) system for the assessment of dry eye patients.

• DESIGN: Prospective comparative study.

• METHODS: Monocular recognition acuity measured continuously by the FVAM system during a 30-second blink-free period was defined as functional visual acuity (FVA). Examinations using the FVAM system were conducted in 35 eyes of 20 healthy controls and 19 eyes of 13 dry eye patients. Tear function examinations including the Schirmer test, tear film break-up time, and fluorescein and Rose Bengal staining were performed in all subjects. Functional visual acuity and tear functions were also examined before and after insertion of punctum plugs in dry eye patients. Functional visual acuity results at 10, 20, and 30 seconds were compared.

• RESULTS: Functional visual acuity in dry eyes were significantly lower than control subjects at all time points ($P < .05$). Functional visual acuity after punctum plugs insertion improved significantly at all time points ($P < .05$).

• CONCLUSIONS: FVAM system seemed not only to be an effective tool in the assessment of dynamic visual acuity changes in dry eye and normal subjects but in evaluating the outcome of management of dry eye disease by punctum plugs. (Am J Ophthalmol 2005;139: 253–258. © 2005 by Elsevier Inc. All rights reserved.)

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DRY EYE PATIENTS OFTEN COMPLAIN OF DECREASED visual acuity during daily activities such as reading, driving and visual display terminal (VDT) work.[1,2] It has been reported that a stable tear film over the corneal surface is essential for clear visual imaging and that irregular corneal surface resulting from dry eyes is associated with poor quality of vision.[1,3,4] Many dry eye patients complain of problems in the quality of vision despite normal visual acuity. Assessment of functional visual acuity (FVA) has been reported to be useful in the detection of such subtle changes in visual quality in dry eye patients.[1,5]

In this study, we performed FVA measurements in dry eye patients, comparing the results with those of normal control subjects and also investigated the changes in FVA with punctum plug treatment.

METHODS

• SUBJECTS: Continuous functional visual acuity measurement was performed in 19 eyes of 13 consecutive dry eye patients (1 male and 12 females; average age 59.6 ± 6.7 years) at Tokyo Dental College, Department of Ophthalmology, Dry eye subspecialty clinic between July 2002 and March 2003. Thirty-five eyes of 20 healthy control subjects also underwent the same examinations (7 males and 13 females; average age 35.0 ± 9.0 years).

Patients with best corrected distance visual acuity less than 20/20 or who had a retinal disease contributing to decreased best-corrected distance visual acuity were excluded from the study. According to these criteria, seven eyes of dry eye patients and five eyes of control subjects were excluded from FVA measurements in this study. This research followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects after explanation of the nature and possible consequences of the study. Ethic committee approval was not required for this study. None of the subjects had any history or evidence of

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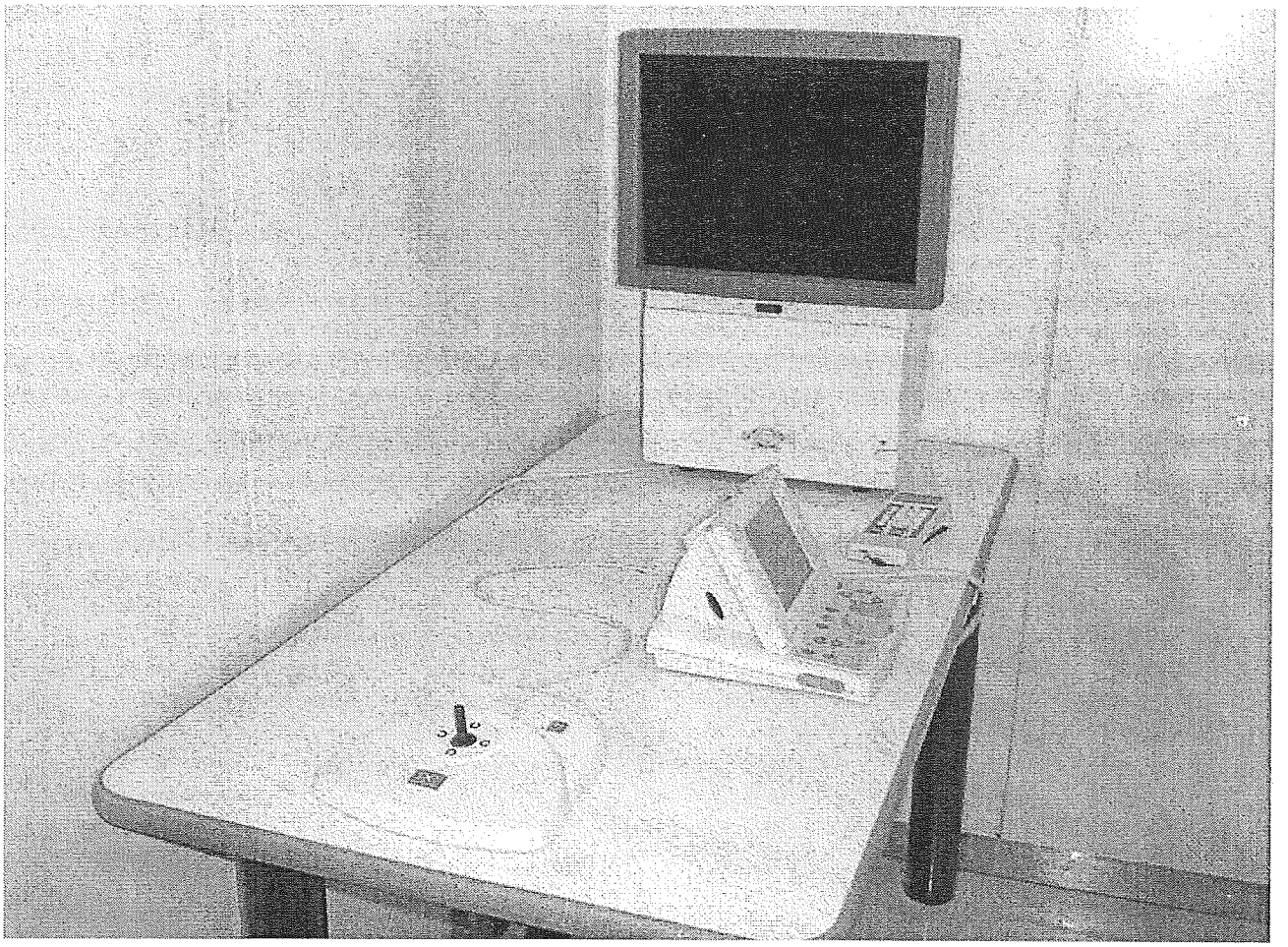

FIGURE 1. The FVAM system. Note that the SSC-350 system is a compact device measuring 56 cm × 39.6 cm × 26.8 cm in size. The optotypes are presented at a distance of 1.1 m from the patients.

TABLE 1. The Tear Film Break-up Time, Schirmer Test, and Ocular Surface Vital Staining Scores in Dry Eye Patients and Control Subjects

	Dry Eye Subjects	Controls	*P* Value
Fluorescein staining (0–9 pts)	3.6 ± 2.2	0.0 ± 0.0	<.05
Rose Bengal staining (0–9 pts)	4.7 ± 2.5	0.0 ± 0.0	<.05
BUT (sec.)	2.6 ± 1.5	6.7 ± 2.1	<.05
Schirmer test (mm)	4.3 ± 1.0	11.3 ± 4.7	<.05

BUT = tear break-up time.

ocular infection, contact lens wear, blepharospasm, abnormal blinking patterns, or had punctum occlusion performed on them previously.

• TEAR FUNCTION DIAGNOSTIC CRITERIA AND OCULAR SURFACE EVALUATIONS: The diagnosis of dry eye was based on the diagnostic criteria of the Dry Eye Research Group in Japan.[6,7] In brief, patients with 1 dry eye-related symptoms, 2 positive staining with fluorescein or Rose Bengal, and 3 Schirmer 1 test results of less than 5 mm, or tear film break-up time (BUT) values of less than 5 seconds were diagnosed as having dry eyes. Subjective symptoms such as sensation of dryness, foreign body sensation, ocular pain, and fatigue were checked using a visual analog scale. Briefly, the visual analog symptom scales were prepared as 10-cm lines, and the patients were asked to check a point on the line corresponding to the degree of their symptom.

The ocular surface was examined by the double vital staining method.[8] Two microliters of preservative-free combination of 1% Rose Bengal and 1% fluorescein dye was instilled in the conjunctival sac. Rose Bengal staining of the ocular surface was scored as described by van Bijsterfeld,[9] and fluorescein staining was scored according to the protocol described by Toda and associates.[8] A fluorescein staining score above 1 point and a Rose Bengal staining score of above 3 points was considered as abnormal. Tear break-up time was measured three times and the mean value was calculated.[8] Sjögren syndrome patients

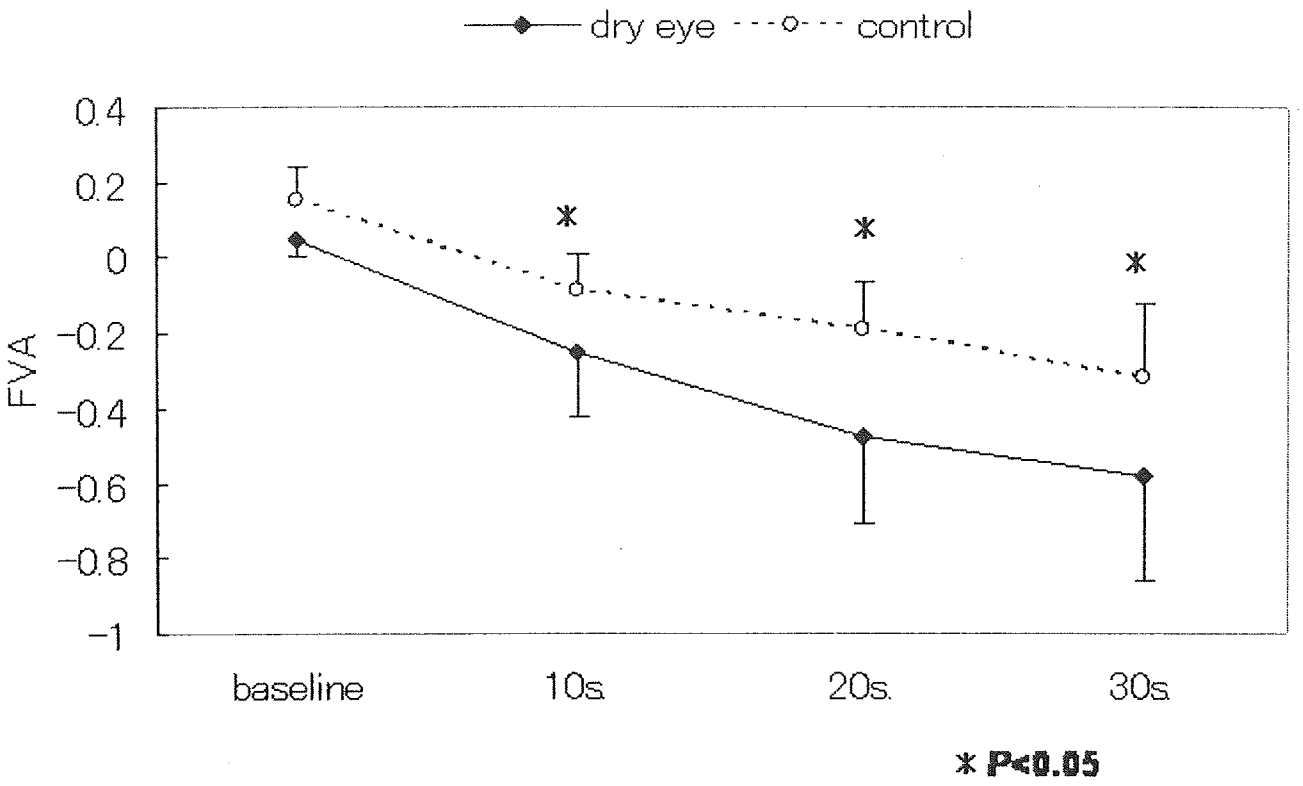

FIGURE 2. The timewise change of FVA in dry eye patients and controls.

were also included and diagnosed by the criteria proposed by Fox.[10] We also recruited healthy control subjects with normal tear function and no vital staining of the ocular surface.

• FUNCTIONAL VISUAL ACUITY MEASUREMENTS: We used the Functional Visual Acuity Measurement System (SSC-350, NIDEK, Gamagori, Japan) to measure the recognition visual acuity continuously (Figure 1). The SSC-350 system is a compact device measuring 56 cm in height and 39.6 cm × 26.8 cm in width. The screen onto which the optotypes are projected within the device measures 25.8 cm × 33.8 cm. The optotypes are presented at a distance of 1.1 m from the patients. Each Landolt optotype presented at a certain visual acuity level within the device subtends an equivalent angle to the optotype of the same visual acuity level presented from 5 m during the conventional Landolt visual acuity testing. We defined the FVA as the monocular recognition acuity measured by the Functional Visual Acuity Measurement System during a 30-second blink-free period. First, visual acuity was measured with no restraints of blinking using this instrument (baseline FVA). Topical anesthesia (0.4% oxybuprocaine chloride) was then administered before FVA examination to minimize discomfort and prevent reflex tearing and blinking. Five minutes after instillation of topical anesthesia, patients were instructed not to blink for 30 seconds during the measurement of FVA. Functional visual acuity at 10 seconds, 20 seconds, and 30 seconds were measured and compared between the patients and control subjects. The examiner also confirmed the absence of blinking during the 30-second period. Patients delineated the orientation of the automatically presented Landolt rings by handling the joystick. Initially, optotypes equivalent to baseline FVA level were presented on the space-saving terminal display at a distance of 1.1m to the subjects. The Landolt ring increased in size automatically when the answer was incorrect. If the Landolt ring was recognized correctly, the same size ring was displayed at random again. The result was recorded as a list of continuous FVA scores with decimal notations.

• PUNCTUM PLUG PROCEDURE: Punctum plugs were inserted in 19 eyes of 13 dry eye patients as described previously in our recent report.[11] Two types of punctum plugs, FCI plug (FCI Ophthalmics, Issy-Les-Moulineaux, France) and Eagleplug (Eagle Vision, Memphis, Tennessee, USA), were used in this study. Sixteen eyes received Eagle plugs, and three eyes had FCI plug insertion. Eagle plugs were preferred for punctal occlusion in eyes with punctal diameters measuring less than 0.8 mm. The three eyes with a punctal diameter of 1.0 mm received FCI plug insertion. Plugs were inserted in both superior and inferior lacrimal puncta in all dry eye subjects. Tear function and

ocular surface examinations were performed before and 2 weeks after the insertion of plugs.

• STATISTICAL ANALYSIS: A two-way repeated-measures analysis of variance test was performed for the comparison of functional visual acuity scores between normal subjects and dry eye patients. The same test was carried out for the comparison of functional visual acuity scores before and after punctum plug insertion. The Mann-Whitney U test was performed for the comparison of tear functions and vital staining scores between normal subjects and dry eye patients.

Wilcoxon's signed rank test was carried out for the comparison of tear functions, vital staining, and subjective dry eye visual analog symptom scores before and after punctum plug treatment. A P value of less than .05 was considered statistically significant. StatView software (SAS Institute Inc, USA) for Windows 98/2000 was used for statistical analysis.

RESULTS

• TEAR FUNCTION ASSESSMENTS IN DRY EYE PATIENTS AND NORMAL SUBJECTS: Six patients had SS, and seven patients had non-SS dry eyes. Dry eye patients had significantly lower Schirmer test, and break-up time scores with significantly higher vital staining scores compared with the control subjects ($P < .05$). The tear functions and vital staining scores of the dry eye subjects and the controls are shown in Table 1.

• FVA IN DRY EYE PATIENTS AND CONTROLS: Mean FVA scores showed a time wise decrease during testing in both dry eye patients and control subjects. The mean FVA scores were significantly lower in dry eye patients than the controls at each time point ($P < .05$). Likewise, the magnitude of the time wise variation over 30 seconds in dry eyes was statistically different from the magnitude of change observed in the control eyes (Figure 2) ($P < .05$)

• TEAR FUNCTIONS AND SYMPTOMS BEFORE AND AFTER PUNCTUM PLUG INSERTION: We did not observe any complications related to punctum plug insertion. The tear functions and vital staining scores of the dry eye patients improved with punctum plug treatment and are shown in Table 2. Likewise, subjective dry eye visual analog symptom scores improved with punctum plug treatment as shown in Table 3.

• COMPARISON OF FVA IN DRY EYE PATIENTS BEFORE AND AFTER PUNCTUM PLUG TREATMENT: Mean FVA scores were observed to be significantly improved with punctum plug treatment at each time point ($P < .05$). Likewise, the magnitude of the time wise variation over 30 seconds after punctum plug treatment was statistically different from the magnitude of change observed before punctal occlusion, as shown in Figure 3 ($P < .05$).

TABLE 2. The Tear Film Break-up Time, Schirmer Test, and Ocular Surface Vital Staining Scores in Dry Eye Patients Before and After Punctum Plug Treatment

	Before	After	*P* Value
Fluorescein staining (0–9)	3.3 ± 2.9	1.9 ± 2.3	<.05
Rose Bengal staining (0–9)	4.2 ± 3.0	3.2 ± 2.6	<.05
BUT (sec.)	2.7 ± 2.0	3.7 ± 1.8	>.05
Schirmer test (mm)	4.9 ± 6.6	7.0 ± 6.9	<.05

BUT = tear break-up time.

TABLE 3. The Change of Subjective Symptom Visual Analog Scale Scores Before and After Punctum Plug Treatment in Dry Eye Patients

	Before	After	*P* Value
Ocular fatigue	2.9 ± 1.5	6.9 ± 2.5	<.05
Dryness	2.5 ± 2.4	6.7 ± 2.7	<.05
FBS	2.7 ± 1.7	5.4 ± 0.9	<.05
Pain	3.0 ± 1.9	6.6 ± 2.5	<0.5

FBS = foreign body sensation.

DISCUSSION

ALTHOUGH STANDARD VISUAL ACUITY TESTING IS AN excellent measure of one aspect of visual function, contrast sensitivity and glare testing provide more important and detailed information on specifics of visual function. Recently, FVA testing described as "functional visual acuity without blinking" has been reported to be an important method of defining "detailed visual function."[1] The method has been shown to be efficient in the detection of "masked impairment of visual function" in dry eye patients who complain of decreased visual acuity despite normal conventional visual acuity test results. The definition of FVA testing has been suggested to be an important indication of an individual's performance in relation to certain daily activities such as driving, reading, and video display terminal works.

Goto and associates previously reported abnormalities of FVA in dry eye subjects.[1] The major drawbacks in that study were the subjectivity of the method of measurement and uncertainty of the timing of FVA measurements, because the visual acuity measurements were carried out using conventional Landolt charts by elevating the patients' upper eyelids manually for 10 to approximately 20 seconds.[5] Moreover, manual elevation of the upper eyelid might have influenced the tear film dynamics and intro-

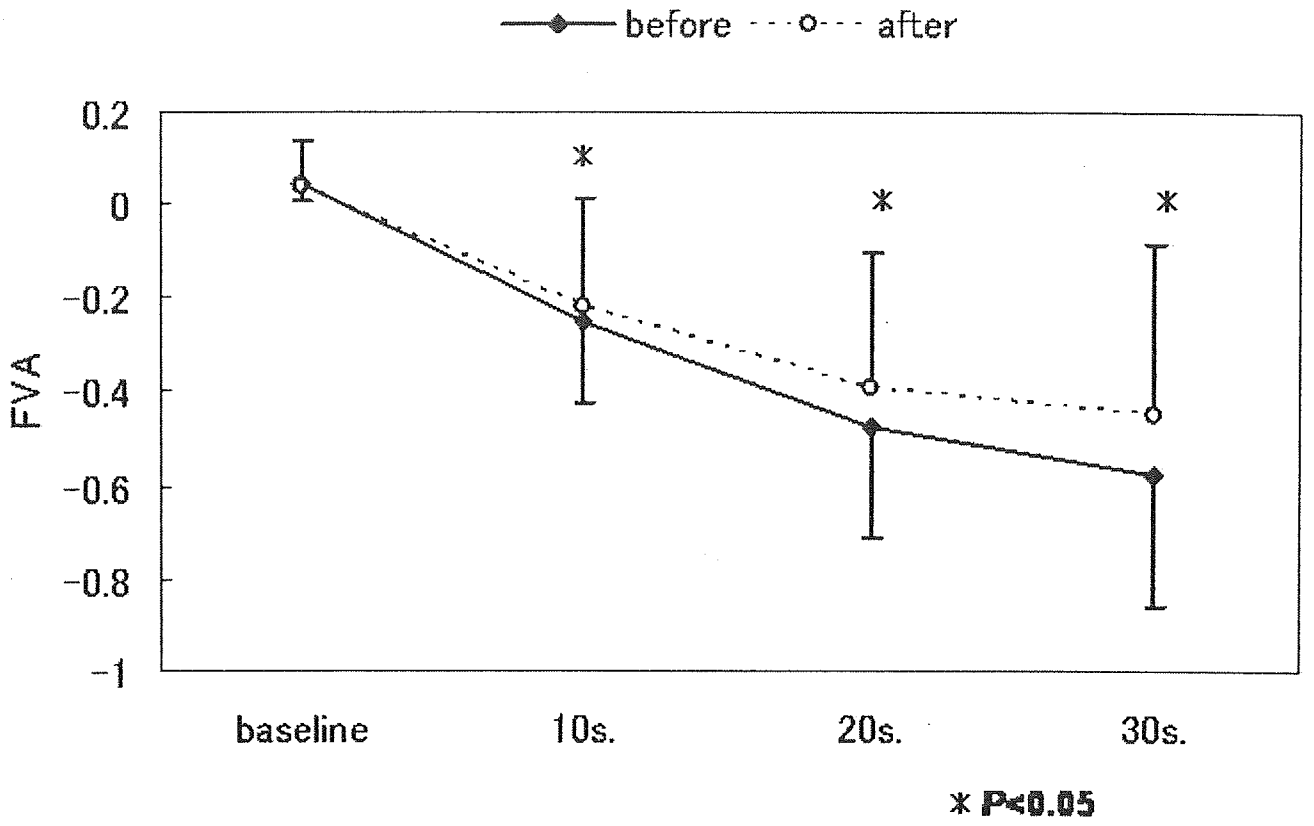

FIGURE 3. The timewise change of FVA with punctum plug treatment in dry eye patients.

duced biases related to evaluation of the effect of natural tear film status on FVA. Functional visual acuity has also been reported to be effective in evaluating dynamic visual function changes after laser-assisted in situ keratomileusis.[12]

We carried out dynamic assessment of visual acuity by performing FVA measurements in dry eye patients, comparing the results with those of normal control subjects and also investigated the changes in FVA with punctum plug treatment. The newly developed FVA device was a compact and a space-saving system that allowed continuous dynamic evaluation of the distance visual acuity. We carried out FVA measurements in the subjects of this study without touching the eyelids, which we believe allowed us to assess the effect of natural tear film status on dynamic visual function.

In this study, the lower visual acuity scores observed at 10, 20, and 30 seconds in dry eye patients compared with the control subjects suggest impaired dynamic visual function on prolonged gaze in dry eye disease. It should be noted that a topical anesthetic agent was used in this study since the FVA measurements were carried out for 30 seconds according to the protocol of the current work. The use of a topical anesthetic agent to reduce discomfort and prevent excessive blinking may introduce new variables of toxicity and tolerance of toxic medications by the ocular surface in dry eye patients. It is our experience that 10 seconds of testing is sufficient to delineate the differences between normal and dry eyes, and we believe that future testing should be performed for 10 seconds without an anesthetic agent. It has been reported that an irregular corneal surface resulting from aqueous deficiency is associated with poor quality of vision.[3-5]

A recent study using a new noninvasive tear stability analysis system, which evaluated the tear stability with dynamic videokeratoscopic images of the tear film captured continuously every second for 10 seconds, employing topographical surface regularity and asymmetry indices, revealed significant degradation of the kinetic tear stability in dry eye patients with worsening of the indices over time. That study also showed improvement of surface regularity and asymmetry indices in dry eye patients who underwent PP occlusion. Although tear stability analysis system measurements were not performed in this study, timewise worsening of corneal surface regularity and asymmetry indices in dry eyes might have resulted in the decreased FVA scores in our dry eye patients.[11]

Our findings that FVA improved in dry eye patients undergoing punctum plug treatment might be explained by improvement in such topographical indices. It should also be noted that the patient and the control groups differed significantly with respect to age and gender characteristics,

which might have influenced the tear function and FVA results in this study. Simultaneous dynamic tear stability analysis system and FVA measurements in dry eye patients are essential and would provide very interesting information. Additionally, studies looking into the correlations between the FVA scores and National Eye Institute or Ocular Surface Disease Index scales would also provide invaluable information.

In summary, the FVAM system seemed not only to be an effective tool in the assessment of dynamic visual acuity changes in dry eye and normal subjects but promising in evaluating the outcome of management of dry eye disease.

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temic antibiotic therapy, it is unclear if the early inflammation represented an allergic reaction or an infection. This is highlighted by the fact that the implant did not need to be removed to achieve clinical improvement as in the two reported cases of orbital cellulitis.[3,4]

Although rare, orbital complications may occur after glaucoma tube shunt surgery. In cases of clear orbital infection, we recommend systemic antibiotic therapy and removal of the implant.

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Tear Film and Ocular Surface Abnormalities After Eyelid Tattooing

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Abstract: **A 45-year-old woman who underwent eyelid tattooing 20 years earlier presented with decreased left vision and eye discomfort. Her ocular history revealed an uneventful LASIK procedure 4 years previously with epithelial problems of the flap followed by *S. aureus* keratitis. Resultant corneal opacity necessitated a deep lamellar keratoplasty performed 9 months prior to admission followed by frequent epithelial problems. At the time of admission, her left eye had a corneal epithelial defect and both lower eyelid margins revealed subepidermal pigmentation and keratinization. Her initial examination revealed tear instability with increased ocular surface staining scores and advanced tear film lipid layer abnormality in both eyes. Meibography showed bilateral total meibomian gland dropout. Treatment with autologous serum eye drops resulted in full epithelialization. Meibomian gland disease-specific therapy did not result in any change in breakup time, vital staining scores, tear film lipid layer interferometry grades, or glandular dropout state.**

A survey of the American Society of Ophthalmic Plastic and Reconstructive Surgery (ASOPRS) membership showed that 42.4% of members performed eyelid tattooing. They encountered mainly technical complications such as pigment fanning, improper pigment placement, and pigment migration in 12.7% of their patients.[1] It is not recommended by ASOPRS members that pigment be placed on the eyelid margin posterior to the eyelashes and in close proximity to the lacrimal punctae.[1] Very little is known about the long-term tissue and ocular surface alterations associated with eyelid tattooing.

CASE REPORT

We examined a 45-year-old Chinese woman with a post-LASIK infectious keratitis OS that had resulted in a generalized corneal opacity. We performed an uneventful deep lamellar keratoplasty. The patient was readmitted 9 months later with complaints of irritation and foreign body sensation OU. Slit lamp examination showed bilateral superficial punctate keratopathy and an inferior corneal epithelial defect in the graft OS (Fig. 1 A) and bilateral keratinization of lower eyelid margins extending onto the palpebral conjunctiva (Fig. 2 A). Tear film and ocular surface examinations performed at that time revealed bilateral tear film instability with a breakup time deficiency type of dry eye. Our discovery of bilateral advanced tear film lipid layer abnormality in the DR-1 tear film lipid layer interferometry (Fig. 3 A,B) prompted us to perform meibography in this patient, which to our surprise revealed bilateral total drop-out and loss of meibomian glandular structures (Fig. 2 B). Treatment with therapeutic contact

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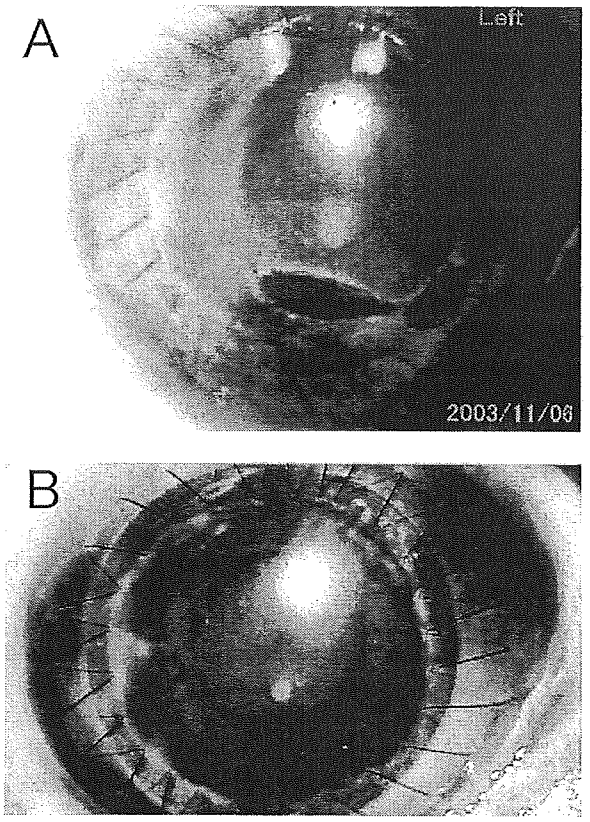

FIG. 1. A. Nine months post deep lamellar keratoplasty, the inferior epithelial defect measured 5×2 mm with surrounding edema and sterile infiltration in the corneal graft OS. **B.** Anterior segment photograph of the corneal graft with complete epithelization at the final examination.

lens and autologous serum drops resulted in complete epithelialization OS after 1 week (Fig. 1 B).

DISCUSSION

Meibomian gland disease (MGD) is associated with the evaporative type of dry eye syndrome and with ocular surface staining.[2] We believe that dry eye symptomatology and ocular surface problems in both eyes owed to the loss of meibomian glands, mechanical effects due to keratinization, and decreased corneal sensitivity in the left eye of our patient. Slit lamp examination showed that blepharopigmentation was carried out posterior to the eyelashes in the vicinity of the meibomian gland orifices. We believe that meibomian gland loss and mucocutaneous junction changes in our patient are associated with blepharopigmentation. She reported a long-term history of irritation in both eyes within a few months after eyelid tattooing. Her ocular history revealed tear instability shortly after the tattooing, with frequent epithelial erosions in both eyes, supporting

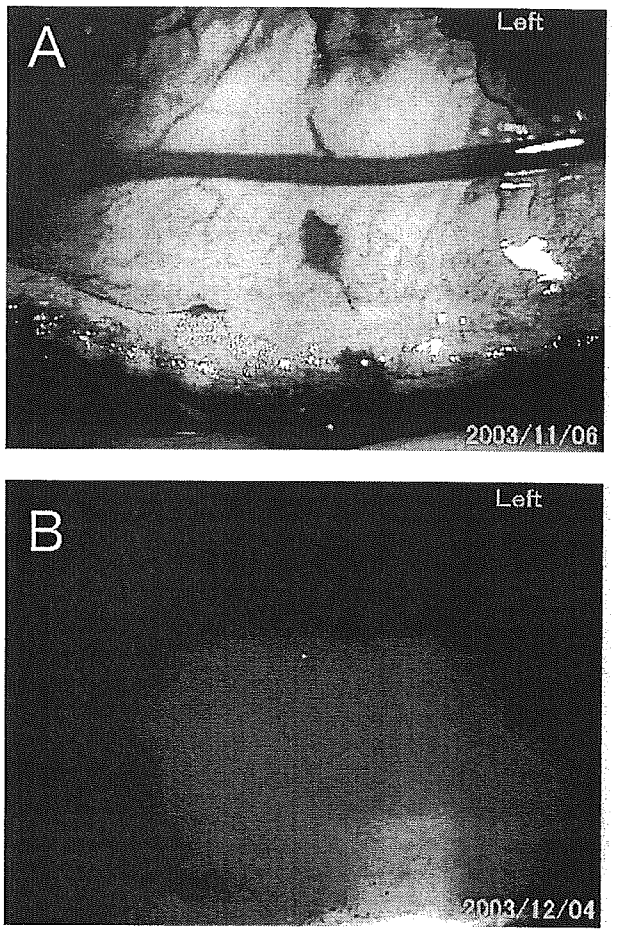

FIG. 2. A. Eyelid margin photograph. Note the subepidermal pigmentation, margin keratinization extending onto the palpebral conjunctiva, cilia loss, and overall obstruction of the meibomian gland orifices. **B.** Note the total loss of meibomian glandular structures and pigment position posterior to lash line around the meibomian gland orifices in the meibography OS.

our belief about the role of blepharopigmentation. In addition, systemic and ocular examination did not reveal other causes of MGD such as acne rosacea, ectrodactyly syndromes, ichthyosis, psoriasis, atopy, blepharitis, chalazia, or trachoma.[2]

Histopathological changes in blepharopigmentation include chronic inflammatory response with sequestration of the pigment in the macrophages, mast cells, and dermal fibroblasts, and foci of elastotic degeneration in the striated muscle and connective tissue.[3–6] To our knowledge, none of the current reports in the literature provide information on gross and ultrastructural changes in the meibomian glands and their implications for the tear film and ocular surface after eyelid tattooing. Meibomian glands are sensitive to inflammation, which may

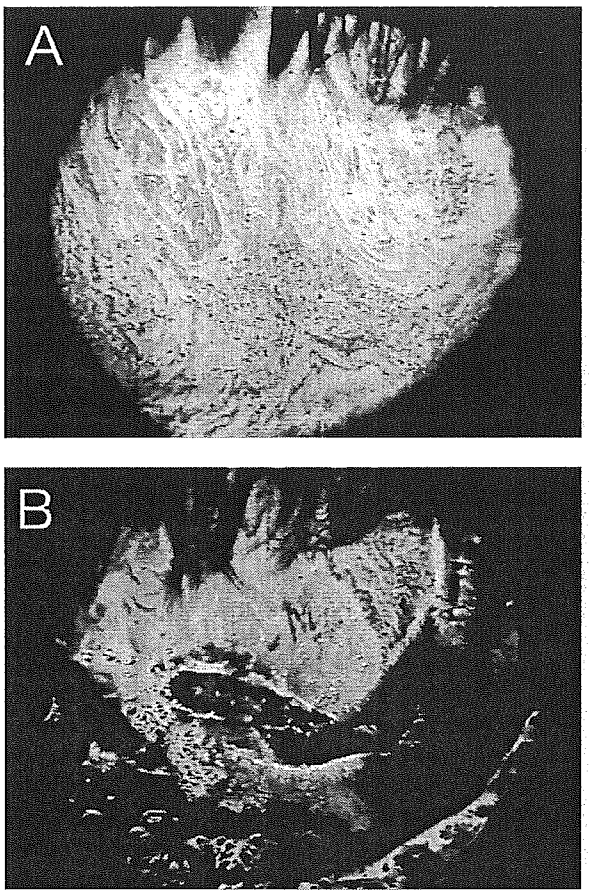

FIG. 3. A. DR-1 tear film lipid layer interferometry print out. Note the advanced lipid layer abnormality with grade 4 dry eye change OD. **B.** DR-1 tear film lipid layer interferometry print out. Note the advanced lipid layer abnormality with grade 5 dry eye changes with areas of corneal exposure over the epithelial defect OS.

cause meibomian glandular dropout.[2] Although we do not have histopathological evidence due to refusal of a biopsy procedure by the patient, we believe that chronic inflammation in addition to improper tattoo technique resulted in extensive gland loss in our patient as evidenced by meibography. We assume that the flap epithelial problems after an uneventful LASIK surgery might have been in part due to evaporative dry eye associated with MGD. In the absence of histopathology to implicate blepharopigmentation as the etiology of evaporative dry eye disease, we looked to the reversibility of the evaporative dry eye state with MGD-specific therapy. Normalization of tear instability and glandular dropout would be expected after treatment of meibomian gland dysfunction associated with blepharitis. In contrast, permanent destruction of meibomian glands by blepharopigmentation should be associated with an irreversible decrease in breakup time, lipid layer interference grades, and glandular dropout. As expected, meibomian gland disease-specific treatment did not improve tear stability, ocular surface staining scores, tear film lipid layer interferometry grades, or glandular dropout status, supporting our theory that blepharopigmentation was responsible for the permanent destruction of the meibomian glands in our patient.

In summary, eyelid tattooing may be associated with extensive meibomian gland dropout, leading to tear film instability and ocular surface epithelial vulnerability, suggesting the need for caution in the practice of eyelid tattooing among patients who are scheduled for LASIK or keratoplasty procedures.

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Sphenocavernous Syndrome Associated With *Schizophyllum commune* Infection of the Sphenoid Sinus

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Abstract: **A 47-year-old diabetic man with chronic renal failure presented with a 1-month history of**

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The Effect of Autologous Serum Eyedrops in the Treatment of Severe Dry Eye Disease: A Prospective Randomized Case-control Study

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• PURPOSE: To evaluate the effectiveness of the autologous serum eyedrops in the treatment of severe dry eye patients.

• DESIGN: Prospective randomized case-control study.

• METHODS: Thirty-seven eyes of twenty severe dry eye patients without punctal occlusion were enrolled in this study. After 2 weeks of washout, they were randomly assigned to two groups. Group A patients used only preservative-free artificial tears, and group S patients used only autologous serum eyedrops. We evaluated the results of Schirmer test, fluorescein and rose bengal staining scores, tear film breakup time (BUT), and subjective symptom scores before and 2 weeks after treatment.

• RESULTS: Mean BUT and fluorescein and rose bengal staining scores, as well as subjective symptom scores, showed significant improvement in the patients assigned to autologous serum eyedrops compared with subjects assigned to preservative-free artificial tears after 2 weeks of treatment.

• CONCLUSIONS: Autologous serum eyedrops were found effective in the treatment of severe dry eye disease, as evidenced by improvement of tear stability and ocular surface vital staining scores. (Am J Ophthalmol 2005; 139:242-246. © 2005 by Elsevier Inc. All rights reserved.)

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CONVENTIONAL TREATMENT OF DRY EYES CONSISTS mainly of the use of preservative-free artificial eyedrops and punctal occlusion.[1] None of the commercially available artificial tear preparations include essential tear components such as epidermal growth factor, hepatocyte growth factor, fibronectin, neurotrophic growth factor, and vitamin A, all of which have been shown to play an important role in the maintenance of ocular surface epithelial milieu. We previously reported that autologous serum eyedrops contain these essential factors and that autologous serum eyedrops were beneficial in the treatment of ocular surface diseases such as persistent epithelial defects, superior limbic keratoconjunctivitis (SLK), keratoconjunctivitis sicca, and neurotrophic keratopathy.[2–7] Comparative controlled studies evaluating the effect of autologous serum treatment on tear function and ocular surface status are still scarce. Studies on autologous serum eyedrops available in the literature do not assess the effects of solitary autologous serum treatment on the ocular surface because of the nature of study designs and presence of punctum plug occlusion in the patients. In this randomized case-control prospective study, we evaluated the solitary effects of autologous serum treatment on the ocular surface health and clinical outcome after a washout period by performing the Schirmer test, BUT, and fluorescein and rose bengal staining scores and comparing the results with those subjects assigned to instillations of preservative-free artificial tear solutions.

METHODS

PATIENT CHARACTERISTICS: Thirty-seven eyes of 20 SS (Sjögren's syndrome) and non-SS patients who matched the following dry eye criteria were enrolled in this study. All patients met the diagnostic criteria of the Japanese Dry Eye Research group[8,9] and had no history of punctal occlusion. Washout was carried out in all subjects with preservative-free saline eyedrops instilled six times a day for 2 weeks. After washout, all patients were randomly

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TABLE 1. Sex, Age, Etiologic Distribution and Baseline Tear Quantities of the Study Population

	Age (years)	Male: Female	SS: Non-SS	Schirmer Test (mm)
Group A	65.4 ± 9.7	2:8	9:1	3.8 ± 4.5
Group S	62.3 ± 12.5	2:8	8:2	3.5 ± 3.1

Group A = patients assigned to artificial tear drops; group S = patients assigned to autologous serum eyedrops; Non-SS = Non-Sjögren's syndrome; SS = Sjögren's syndrome.

assigned to two groups: patients in group A used only preservative-free artificial tears, and patients in group S used only autologous serum drops six times a day for 2 weeks. Ten patients were assigned to each group. There were no statistically significant age and sex differences between the control subjects and patients in this study. The examiner who carried out the tear function and ocular surface evaluations was masked to the type of the eyedrops prescribed to the patients in this study. The research followed the tenets of the Declaration of Helsinki, and informed consent was obtained from all the subjects after explanation of the nature and possible consequences of the study.

VISUAL ANALOG PAIN SYMPTOM SCORE: Absence of any pain constituted a score of 0 points on the visual analog pain scales, and intense, unbearable pain was considered a full pain score of 100 points. Briefly, the visual analog pain symptom scales were prepared as 10-cm lines and the patients were asked to check a point on the line corresponding to their degree of pain.

TEAR FUNCTION TESTS: Tear film BUT was measured three times, and the mean value was calculated.[10] The Schirmer test was performed using standard strips (Alcon, Fort Worth, Texas, USA) kept in the lower conjunctival sac for 5 minutes.

OCULAR SURFACE VITAL STAINING: The ocular surface was examined by the double vital staining method.[10] Two milliliters of a preservative-free combination of 1% rose bengal and 1% fluorescein dye was instilled in the conjunctival sac. Rose bengal staining of the ocular surface was scored according to the criteria proposed by van Bijsterveld.[10,11] The fluorescein staining was scored according to the protocol described by Shimmura and associates[12] Briefly, the cornea was divided into three equal areas of upper, middle, and inferior corneal compartments. Each compartment was graded on a scale of 0 (no staining) to 3 points (intense staining). A fluorescein staining score above 1 point was considered as abnormal (maximum: 9 points). The diagnosis of dry eye was based on the diagnostic criteria of the Dry Eye Research Group in Japan.[8,9] In brief, patients with[1] dry-eye-related symptoms,[2] positive staining with fluorescein or rose bengal, and[3] Schirmer 1 test results of less than 5 mm or tear film BUT values of less than 5 seconds were diagnosed as having dry eyes. Dry eye cases were categorized as non-SS and SS on the basis of the criteria proposed by Fox and associates.[13] Briefly, patients who had dry eyes and dry mouth, serum rheumatoid factor, and antinuclear antibody levels 1:160 or greater, positive serology for anti-SS-A or anti-SS-B antibodies and labial salivary gland inflammatory infiltration focus score of 2 or more were diagnosed as SS. The patients did not have any history of ocular surgery including punctal occlusion, ocular or systemic disease, or a history of drug or contact lens use that would alter the ocular surface. According to the study protocol, tear film BUT analysis was performed initially, followed by fluorescein and rose bengal vital staining of the ocular surface. The Schirmer 1 test was then performed. Serum drops were applied six times a day for 2 weeks. Tear film BUT, vital staining of the ocular surface, and visual analog pain symptom scores were compared before and after treatment.

PREPARATION AND APPLICATION OF AUTOLOGOUS SERUM: We prepared autologous serum eyedrops according to a previously reported protocol by Tsubota and associates.[2] Briefly, a total of 40 ml of blood was procured by venopuncture and centrifuged for 5 minutes at 1,500 rpm. The serum was carefully separated in a sterile manner and diluted to 20% by saline. The final preparation was aliquoted into 5-ml vials with ultraviolet light protection, because vitamin A is easily degraded by light. Patients were instructed to keep the vials they were using in a refrigerator at 4 C and were instructed to store the other vials in a freezer until required.

STATISTICAL ANALYSES: The nonparametric Mann-Whitney *U* test was chosen to compare the unpaired noncontinous data at different time points. The Mann-Whitney *U* test was performed for the comparison of vital staining scores, BUT, and subjective symptom score differences between the two groups. The Chi-square test was applied to test the age and sex differences between group A and S. A *P* value less than .05 was considered statistically significant. StatView software (SAS Institute, Cary, North Carolina, USA) for Windows 98/2000 was used for statistical analysis.

RESULTS

PATIENTS CHARACTERISTICS: Seventeen patients had SS and 3 patients had non-SS dry eyes in this study. Patient characteristics are summarized in Table 1.

SCHIRMER TEST: The mean Schirmer test scores did not vary significantly between the two groups before

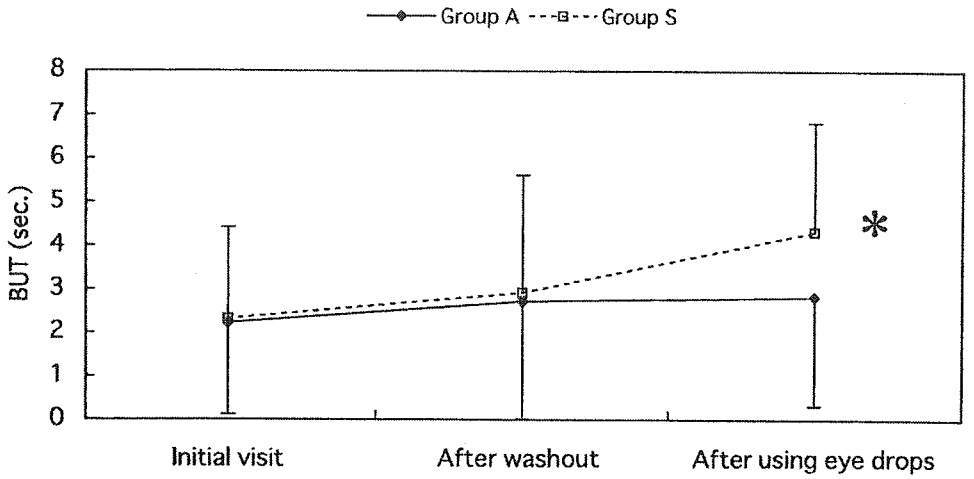

FIGURE 1. Comparison of tear film breakup time between patients in group A (artificial tear) and patients in group S (autologous serum). Breakup time was significantly longer in group S patients compared with the group A patients after treatment ($P < .05$).

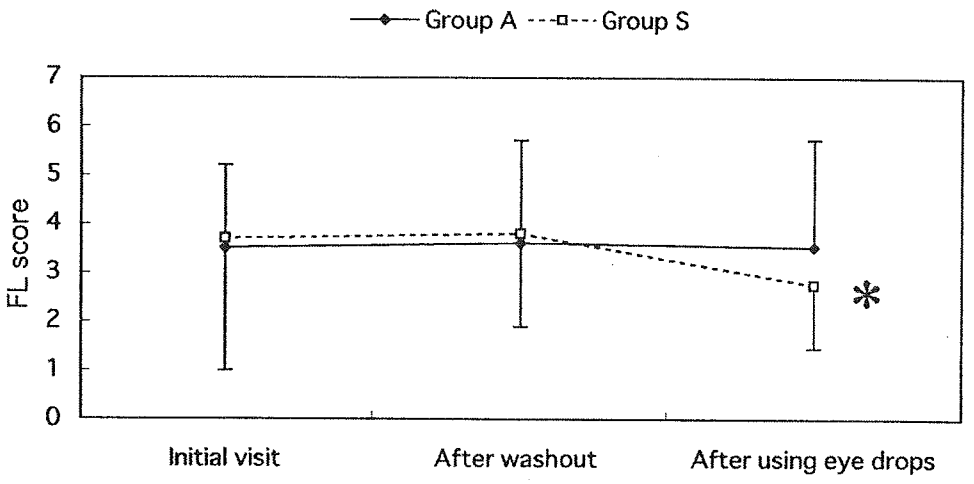

FIGURE 2. Comparison of fluorescein score between patients in group A (artificial tear) and patients in group S (autologous serum). Fluorescein score was significantly lower in group S patients compared with the group A patients after treatment ($P < .05$).

treatment, as shown in Table 1. The final mean Schirmer scores were 3.7 ± 3.1 mm in group A and 3.3 ± 2.6 mm in group S ($P > .05$), respectively. There were no differences between pre- and posttreatment Schirmer test values in each group, and the mean post-treatment values between the two groups.

TEAR FILM BUT: There were no significant differences between the mean BUT values at the initial visit and after washout between the two groups ($P > .05$). The mean BUT value was 2.3 ± 2.3 seconds before treatment with autologous serum eyedrops and 4.3 ± 2.6 seconds after treatment. The mean BUT value in the group treated with autologous serum eyedrops was significantly higher at the end of treatment compared with the group assigned to treatment with artificial tears, as shown in Figure 1 ($P < .05$).

OCULAR SURFACE VITAL STAINING SCORES: The mean fluorescein and rose bengal staining scores did not show significant differences at the initial visit or after washout between the two groups, as shown in Figures 2 and 3 ($P > .05$). The mean fluorescein staining score was 2.8 ± 1.3 points after treatment with autologous serum and 3.5 ± 2.2 points after artificial tear eyedrops. Mean rose bengal staining score was 2.5 ± 2.0 points after treatment with autologous serum and 4.2 ± 2.5 points after treatment with artificial tear eyedrops. The differences in the mean ocular surface staining scores between the two groups after treatment were statistically significant ($P < .05$).

VISUAL ANALOG OCULAR PAIN SYMPTOM SCORES: There were no statistically significant differences related to the visual analog ocular pain symptom

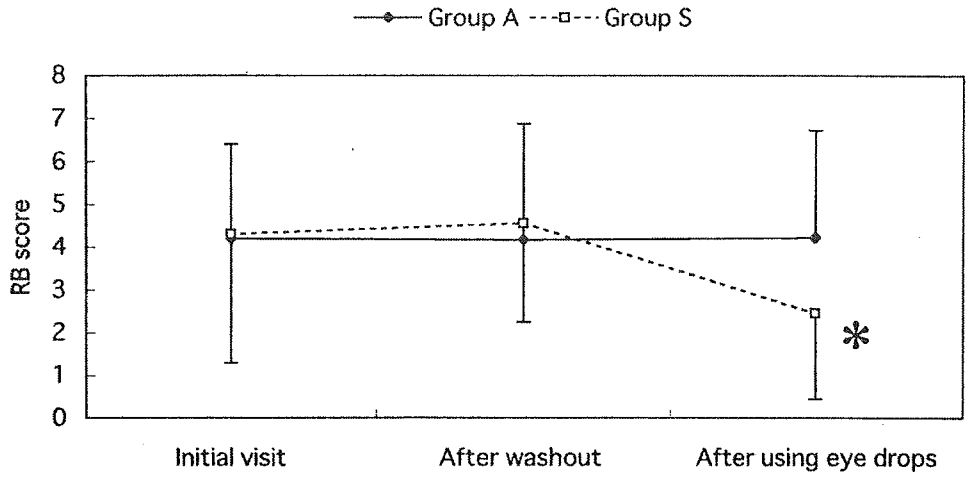

FIGURE 3. Comparison of rose bengal score between patients in group A (artificial tear) and patients in group S (autologous serum). Rose bengal score was significantly lower in group S patients compared with group A patients after treatment ($P < .05$).

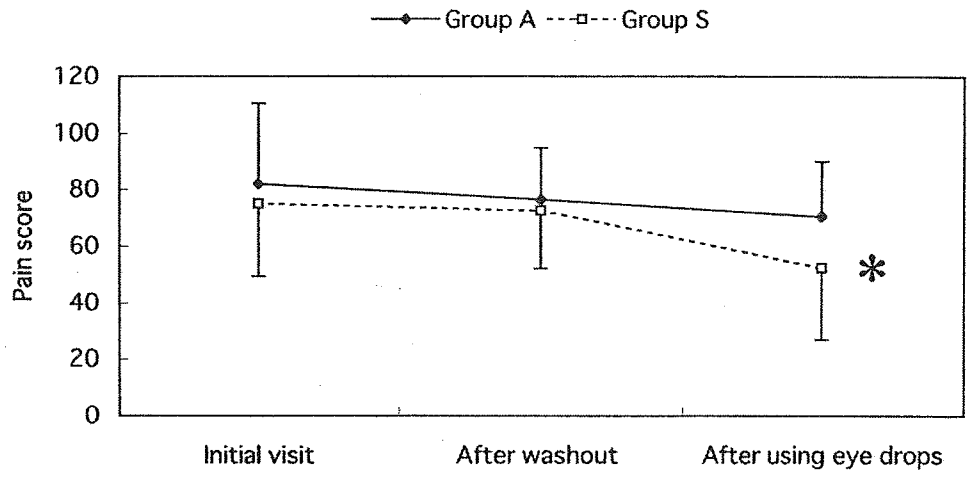

FIGURE 4. Comparison of pain score between patients in group A (artificial tear) and patients in group S (autologous serum). Pain score measured by visual analog scale was significantly lower in group S patients compared with group A patients after treatment ($P < .05$).

scores at the initial visit and after washout between the two groups ($P > .05$). The mean visual analog pain symptom scores were 70 ± 20 points after treatment with autologous serum and 52 ± 24 points after treatment with artificial tear eyedrops. Pain scores were significantly lower in patients assigned to treatment with autologous serum eyedrops compared with patients assigned to artificial tears at the end of 2 weeks ($P < .05$), as shown in Figure 4.

DISCUSSION

DESPITE MAXIMAL CONVENTIONAL TREATMENT, A COhort of severe dry eye patients exists that has persistent symptoms and signs. Such patients have more serious ocular surface disorder, with significant visual impairment and disability.[14] Our previous experience in SLK,[4] recurrent corneal erosions,[2] neurotrophic keratopathy, Sjögren's syndrome[3] and other reports[6,15] in uncontrolled trials supported the benefits of autologous serum eyedrops in these disorders, with improvement of ocular health and tear functions. Tananuvat and associates[16] reported in a controlled trial comparing autologous serum eyedrops in one eye with the use of artificial tears in the fellow eye as a control that there was a statistically nonsignificant trend toward improvement of vital staining scores and tear stability in both eyes. Most eyes in that study had received punctal occlusion, however, which might have interfered with the evaluation of the solitary effects of autologous serum eyedrops and artificial tear eyedrops; this may have caused a bias toward overevaluation of the effects of solitary artificial tears.

A recent randomized controlled clinical trial by Noble and associates[17] assigned severe dry eye patients to either 3

months of autologous serum 50% eyedrops followed by 3 months of conventional treatment with artificial tears or 3 months of conventional treatment followed by 3 months of autologous serum. Use of autologous serum was associated with improvement of impression cytology parameters in that study without any differences found for rose bengal staining, Schirmer test, or tear clearance test. The crossover design of the study confirmed that these improvements were due to serum drops, that the effect was reversed when treatment was reversed, and that the beneficial effects were probably due to the presence of essential tear factors in the serum. Washout was not performed in that study, however, and it included patients who had lacrimal punctal occlusion, which may have resulted in overestimation of the effects of artificial tear drops.

In our study, none of the patients had punctal occlusion, which allowed us to evaluate the solitary effect of the autologous serum drops. We conducted this randomized prospective controlled clinical trial with a 2-week washout and assigned patients into two groups, using only autologous serum or artificial tears. We think this allowed us to evaluate the individual effects of these eyedrops.

We found significant improvements in tear stability, ocular surface vital staining scores, and pain symptom scores in patients treated with autologous serum eyedrops compared with those assigned to nonpreserved artificial tears. Confirmation of our findings with a larger group of patients in additional prospective controlled trials is desirable. Comparative prospective studies on the additive effects of sodium hyaluronate eyedrops and other conventional treatment modalities combined with autologous serum eyedrops would also provide interesting information. Indeed, hyaluroluronate alone has been reported to be effective in improving the corneal epithelial barrier function and in reducing the ocular surface damage in dry eye patients.[18,19] The viscoelastic properties of hyaluronate might result in a longer exposure of the ocular surface to the essential autologous serum components such as growth factors and retinoids. Serum drops were used for 2 weeks in this study. Studies clarifying the effect and risk of prolonged application or of different concentrations of autologous serum drops to the ocular surface should be the subjects of future investigations.

In summary, we found autologous serum to be superior to conventional treatment with artificial tears for improving ocular surface health and subjective comfort.

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Collagen-Immobilized Poly(Vinyl Alcohol) as an Artificial Cornea Scaffold that Supports a Stratified Corneal Epithelium

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Abstract: The cornea is a transparent tissue of the eye, which is responsible for the refraction of incoming light. Both biological corneal equivalents and synthetic keratoprostheses have been developed to replace donor tissue as a means to restore vision. However, both designs have drawbacks in terms of stability and biocompatibility. Clinically available synthetic devices do not support an intact epithelium, which poses a risk of microbial infection or protrusion of the prosthesis. In the present study, type I collagen was immobilized onto poly(vinyl alcohol) (PVA-COL) as a possible artificial cornea scaffold that can sustain a functional corneal epithelium. Human and rabbit corneal epithelial cells were air-lift cultured with 3T3 feeder fibroblasts to form a stratified epithelial layer on PVA-COL. The epithelial sheet expressed keratin 3/12 differentiation markers, the tight junction protein occludin, and had characteristic microvilli structures on transmission electron microscopy. Functionally, the stratified epithelium contained normal glycogen levels, and an apical tight-junction network was observed to exclude the diffusion of horseradish peroxidase. Furthermore, the epithelium-PVA-COL composite was suturable in the rabbit cornea, suggesting the possibility of using PVA-COL as a biocompatible material for keratoprosthesis. © 2005 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 76B: 56–63, 2006

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Corneal transplantation (penetrating keratoplasty, lamellar keratoplasty, and deep lamellar keratoplasty) replaces the diseased recipient cornea with an optically clear donor. A wide variety of corneal disorders are treated by transplantation, and the success rate is high compared to other tissue transplants. However, many countries suffer from a shortage of donor corneas, and the development of an artificial cornea may be a solution to this problem.[1] One strategy for the development of an artificial cornea uses biomaterials to recreate the cornea to produce what is called a corneal equivalent.[2,3] Recent studies have used hybrid polymers that combine both artificial polymers and biological molecules to design scaffolds that can support cell growth.[4,5]

Another strategy uses artificial polymers to design devices that are generally referred to as keratoprostheses (KPros). The ideal material for a keratoprosthesis should not cause excessive inflammation or activation of resident keratocytes, both of which may cause scarring and opacification of the optical interface. Permeability of nutrients from the anterior chamber is also vital to prevent stroma melting and epithelial defects. Although an overlying epithelium is not required in several KPros already available,[6–11] an intact epithelial sheet will serve to stabilize the tear film and prevent extrusion and infection, which are complications observed with Kpros.[12–14]

One candidate material for the KPro is poly(vinyl alcohol) (PVA).[13,15] Low-temperature–crystallized PVA is transparent and has mechanical properties suitable for Kpros.[16] Although pure PVA shows low cell affinity, covalent immobilization of collagen type I to the surface of PVA may improve this point. The present study shows how PVA-COL combined with air-lift organotypic culture techniques support a fully stratified corneal epithelium, which show similar physiological and morphological characteristics with the native cornea.

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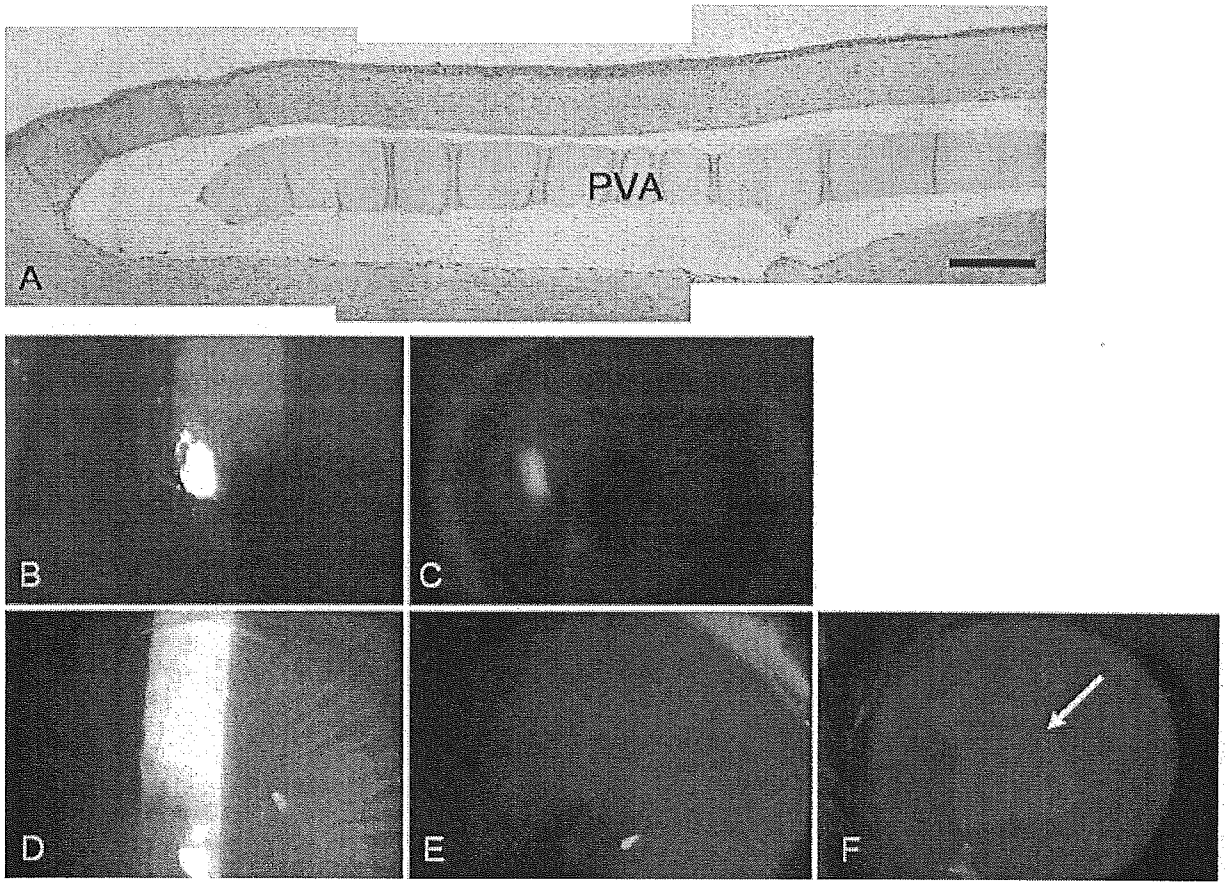

Figure 1. Rabbit cornea after PVA-COL implantation in the stroma. (A) Paraffin section of rabbit cornea at 4 weeks after implantation. Slit lamp photograph (B,D) and fluorescein staining (C,E) of implanted cornea at Day 0 (B,C) and 4 weeks (D,E) after implantation. (F) Fluorescein staining of rabbit cornea implanted with PTFE showing an epithelial defect (arrow). Bar = 200 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

MATERIALS AND METHODS

Immobilization of Collagen onto PVA Hydrogel

PVA-COL hydrogel was prepared as described previously.[13,17] Briefly, PVA powder was dissolved in a dimethyl sulfoxide–water (80–20) mixed solvent. The resulting viscous solution was allowed to stand at −20°C for 24 h for setting a gel. The PVA hydrogel was dehydrated for further surface modification. Isocyanate groups were first introduced onto the surface by using hexamethylene dissocyantate (HMDI). The activated PVA was immersed in type I collagen solution (porcine skin, 0.5 mg/mL, Nitta Gelatin Co. Ltd., Osaka, Japan) to immobilize the collagen on the surface of the PVA. PVA-COL was then washed successively with phosphate-buffered saline PBS (−) with the use of an ultrasonic cleaner for 10 min in order to remove the absorbed collagen, and sterilized by UV irradiation. The amount of collagen immobilized was determined by the bicinchoninic acid (BCA) protein assay by measuring the absorbance (562 nm) with a multi-plate reader, GENios, TECAN Japan Co. Ltd, Japan. From the result, it was found that approximately 0.5 $\mu g/cm^2$ of collagen was covalently immobilized on the surface.

Intracorneal Implants

PVA-COL polymers were transplanted into the right eye of Japanese white rabbits (female, 3-kg body weight, Shiraishi Experimental Animal Breeding Farm, Tokyo, Japan) according to the ARVO Statement for the Use of Animals in Ophthalmology and Vision Research. Recipient animals were anesthetized with 4 mL intramuscular ketamine and xylazine (1:7 mixture) and topical xylocain. Six-millimeter circular stromal pockets were made from the limbus, and a 6-mm-diameter PVA hydrogel was inserted into the pocket ($n = 4$). Polytetrafluoroethene (PTFE) sheet (Teflon sheets, 17-97-07 TGK Co. Ltd., Japan) was purified by Soxhlet extraction with ethanol and was used as positive control ($n = 1$), and an untreated animal ($n = 1$) was used as a negative control. All animals received topical antibiotics (levofloxacin) and steroids (betamethasone) twice a day following surgery. Transplanted eyes were followed by slit lamp examination for up to 1 month, after which animals were sacrificed for histology. The recipient cornea was fixed with 4% paraformaldehyde in PBS at 4°C overnight, and embedded in paraffin. Four–six-micrometer sections were stained with hematoxylin and eosin (HE) and PAS reaction to observe glycogen